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Oxidative stability index of vegetable oils in binary mixtures with meadowfoam oil

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Abstract

The oxidative stability indices (OSI) of several vegetable oils were determined at 110°C. Meadowfoam oil, *Limnanthes alba*, was found to be the most stable oil with an OSI time of 67.3 h for refined oil and 246.9 h for crude oil. Other oils with good oxidative stabilities were refined high oleic sunflower and crude jojoba oil (*Simmondsia chinensis*) with OSI times of 49.8 and 34.5 h respectively. The unusually high OSI time of crude meadowfoam oil could not be attributed to its tocopherol content since refining did not significantly alter the tocopherol content, but significantly reduced the stability. A relationship of iodine value to antioxidant was developed for vegetable oils; however, this linear relationship did not account for the unusually high oxidative stability of meadowfoam oil. Binary mixtures of vegetable oils were also examined for enhanced oxidative stability. Small amounts of crude meadowfoam oil gave enhanced oxidative stability in mixtures with jojoba, triolein and castor oils. Triolein/crude meadowfoam oil mixtures showed the most dramatic improvements in OSI time with a 5% (w/w) addition of crude meadowfoam causing a 21-fold increase in the OSI time. Meadowfoam mixtures with jojoba improved the OSI time of jojoba from 31.1 to 52.7 h, when 10% crude meadowfoam oil was added. A study of oxidative stability with respect to olefin position showed that the Δ5 double bond was the most stable by more than an order of magnitude. The oxidative stability of meadowfoam FAMEs at 90°C gave an OSI time of 4.9 h which was the same as methyl erucate and methyl petroselenate, but less than methyl oleate which had an OSI time of 14.8 h. However, when methyl 5-eicosenoate was isolated in high purity (monoene > 96%) and tested, it gave an OSI time of 69.4 h.

Keywords: Oxidative stability index; Meadowfoam oil; Vegetable oils; 5-Eicosenoic acid; Fatty acids; Binary oil mixtures

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1. Introduction

A recent review (Kamal-Eldin and Appelqvist, 1996) on the chemistry of antioxidants summarized a large body of research on the role of tocopherols in lipid oxidation. However, the continued poor oxidative stability of vegetable oils, even with their natural antioxidants present, often failed to meet the rigorous demands of cosmetic, food and industrial applications. Because of the poor oxidative performance of vegetable oils, alternative and new potential antioxidants continue to be sought (Duh and Yen, 1997). One avenue that has not been thoroughly explored is the utilization of new crop seed oils in blends with less stable traditional oils. Several new crop oils such as jojoba and meadowfoam have superior oxidative stability compared to traditional vegetable oils. High oleic sunflower with an AOM value of 90 (Fitch, 1994), meadowfoam oil with an AOM of 153 (Muuse et al., 1992), and jojoba oil are good candidates for the development of binary oil mixtures with potentially good oxidative stability. This study looks at binary mixtures of traditional vegetable oils with new crop oils and how such admixtures impact the oxidative stability of the triglyceride.

A good method for following the stability of vegetable oils is the oxidative stability index (OSI), which determines the oxidative stability of an oil by passing air through a sample under stringent temperature control (Firestone, 1993a). A stream of air is passed through the oil sample, which aids in the rapid degradation of the triglyceride into volatile organic acids. The air stream flushes the volatile acids from the oil into a conductivity cell containing water where the acids are solubilized. These acids, once dissolved in the water solution, disassociate into ions, thus changing the conductivity of the water. Therefore, a continuous measure of the conductivity of the cell by computer will indicate when a rapid rise in the conductivity occurs that corresponds to the induction point, oxidative failure of the sample. The time to the induction point is the OSI time. An AOCS standard method has been recently developed and a collaborative study has also been published (Jebe et al., 1993) demonstrating that the OSI method has good reproducibility among samples and laboratories.

The tocopherol content of an oil has a dramatic impact on its oxidative stability (Kamal-Eldin and Appelqvist, 1996) and there is general agreement as to the order of oxidative protection by tocopherols is $\alpha > \beta = \gamma > \delta$. We determined the total amount of tocopherol in the oils and used this value as a marker for the anti-oxidant available to protect the oil. However, we remain fully aware of the potential for other minor components that may be present in the oils and serve as antioxidants. With these concepts in mind, we noted a good relationship in a recent article by Matthaus (1996) that reported total tocopherol for several oils and their rancimat oxidative stabilities along with their iodine values. The ratio of iodine value to tocopherol concentration from Matthaus' data gave a linear relationship with respect to oxidative stability. This relationship held up for a diverse set of vegetable oils that we examined and provided a baseline for determining synergistic relationships that developed in binary mixtures.

This study also provides a preliminary look at the oxidative stability of monoenoic fatty methyl esters with respect to double bond position in the absence of anti-oxidants. This contrasts with earlier studies that examined the stability of methyl esters as mixtures (Takagi and Miyashita, 1987; Kaneniwa et al., 1988). We found that the 5-olefinic position possess unique stability when compared to Δ6, Δ9 and Δ13 olefins that we examined.

2. Materials and methods

All triglycerides and wax esters were used as provided by the manufacturer and stored at −10°C until used. Refined and unrefined meadowfoam oil and castor oil were provided by The Fanning Corp. (Chicago, IL). Refined jojoba oil, coriander oil and high oleic sunflower were provided by International Flora Technologies, (Gilbert, AZ). High erucic acid rapeseed oil was supplied by Calgene Chemical (Skokie, IL). Technical grade triolein (65%) was obtained from MCB (Norwood, OH) and Sigma (St. Louis,

MO). Oleic acid (90%) was obtained from Aldrich (Milwaukee, WI). Triolein (95 and 99+%) and tocopherol standards were also obtained from Sigma. High pressure liquid chromatograph (HPLC) solvents hexane and acetone were purchased from Fisher Scientific (Fair Lawn, NJ). Saturated fatty acid methyl ester (FAME) standards were obtained from Alltech Associates (Deerfield, IL).

OSI determinations were performed on a oxidative stability instrument manufactured by Omnion (Rockland, MA) using the AOCS method Cd12b-92 (Firestone, 1993a). Triglycerides and jojoba oil samples were run at 110°C and FAMEs were tested at 90°C. Air flow was set at 35 kPa with a resulting velocity of 140 ± 8 ml/min. Samples were massed to 5.00 ± 0.05 g for single oil analyses. For binary oil systems the minor oil component was massed to four decimal places based on the percentages reported in Table 3 and the bulk solution brought to 5.00 ± 0.05 g. All OSI samples were run in replicate and Table 1 gives the standard deviation data for the parent oils. Table 3 provides data for the admixtures with relative standard deviations less than 2.0 for replicate runs ($n \geq 2$). Outliers in the data set were eliminated by the students Q-test. The degree of variance within the data set (Tables 2 and 3) is below that of a collaborative study (Jebe et al., 1993), where intralaboratory variation was 3.21% for an OSI instrument. The conductivity probes were cleaned by the method of Akoh (1994). In addition, each run used new tygon tubing to direct air flow and a new borosilicate tube was used for each sample. OSI times were determined by the second derivative method which provided the best relative standard deviations compared to the tangent method or values reported directly from the OSI software package.

Gas chromatography (GC) was performed with a Hewlett-Packard 5890 Series II gas chromatograph (Palo Alto, CA) equipped with a flame ionization detector and an autosampler/injector. Analyses were conducted on two columns: an SP 2380, 30 m × 0.25 mm i.d. (Supelco, Bellefonte) and a SGE BP-1, 30 m × 0.22 mm i.d. (Scientific Glass Engineering, Austin). Saturated C8-C30 FAMEs provided standards for calculating equivalent chain length (ECL) values, which were used to make FAMEs assignments.

SP 2380 analysis: column flow 1.2 ml/min with a helium head pressure of 15 psi; split ratio 40:1; programmed ramp 150–175°C at 2°C/min, 175–220°C at 5°C/min and 220–265 at 20°C/min; injector and detector temperatures set at 250°C. BP-1 analysis conditions: column flow 1.2 ml/min with a helium head pressure of 15 psi; split ratio 40:1; programmed ramp 175–265°C at 3°C/min; injector and detector temperatures set at 250°C.

Iodine values were then calculated from each column's results and averaged using AOCS method Cd 1c-85 (Firestone, 1993b).

Table 1
OSI values of parent oils used in binary oil studies

Oil	OSI at 110°C	No. of replicates	S.D.
Crude meadowfoam oil (CMFO)	246.9	3	2.54
Refined meadowfoam oil (RMFO)	67.3	6	1.33
Cold pressed jojoba oil (PJO)	55.9	2	0.98
Refined high oleic sunflower (RHOSO)	49.8	5	0.11
Refined castor oil (RCAS)	56.1	2	0.14
Crude jojoba oil (CJO)	34.5	2	1.21
Refined jojoba oil (RJO)	31.4	2	0.05
Deodorized jojoba oil (DJO)	23.5	3	0.97
Refined soybean oil (RSO)	19.9	2	0.00
Reagent grade triolein (T65)	8.5	10	0.17
High erucic acid rapeseed (HEAR)	8.1	2	0.12
Triolein 99+% (T99)	2.7	2	0.03
Triolein 95% (T95)	1.8	2	0.12

Table 2
OSI values of binary oil systems at 110°C

Ratio	T-99	T-95	T-65	T-65	T-65	T-65	T-65	RHOSO	RJO	HEAR	RCAS	RSO
	CMFO	CMFO	CMFO	RMFO	RHOSO	CJO	RJO	CMFO	CMFO	CMFO	CMFO	CMFO
100	2.7	1.8	8.5	8.5	8.5	8.5	8.5	49.8	31.3	8.1	82.9	19.9
95:5	51.5	38.5	11.5	9.9	9.9	9.8	8.7	49.9	43.6	NA	90.0	19.9
90:10	76.1	52.1	13.5	9.9	9.9	9.9	9.1	51.3	52.7	8.6	102.9	19.9
80:20	NA	NA	17.3	11.7	12.0	11.3	9.9	52.2	72.2	10.0	134.7	19.9
70:30	NA	NA	22.8	13.0	13.7	12.0	9.9	NA	NA	12.2	NA	NA
60:40	141.2	152.7	32.8	16.8	16.2	14.1	11.8	64.9	109.9	14.3	175.0	19.9
0	246.9	246.9	246.9	67.3	49.8	34.5	31.4	246.9	246.9	246.9	246.9	246.9

Top oil is the parent and the bottom oil is the blend at the ratio listed in the first column.
T99, triolein 99+% pure; T95, triolein 95% pure; T65, technical grade triolein 65% pure.
CMFO, crude meadowfoam oil; RMFO, refined meadowfoam oil; CJO, crude jojoba oil; RHOSO, refined high oleic sunflower oil; RJO, refined jojoba oil; HEAR, high erucic acid rapeseed oil; RCAS, refined castor oil; RSO, refined soybean oil.

HPLC analyses were performed on a Thermo Separation Products instrument with a P2000 binary pump, AS2000 autosampler/injector (Fremont, CA) coupled to a UV 2000 ultraviolet detector set at 295 nm. An Alltech (250 mm × 4.6 mm, 60 Å, 8 μm) amino column was used to separate the tocopherol mixtures. Components were eluted from the column with a hexane/dioxane 90:10 mixture at a flow rate of 1 ml/min according to the method of Abidi and Mounts (1996). Standard curves for α-,β-,γ-,δ-tocopherol and ξ-tocotrienol were established. All oil samples were dissolved in hexane at 1:10 oil to hexane ratio and run in duplicate using a 10 μl injection volume.

Triglycerides were converted to their FAMEs by treatment of the oil ($\approx$ 10 mg) with 0.5 ml of 0.5 M KOH/MeOH for 1 h in a sealed vial at 100°C. The vial was cooled to room temperature and 1 ml of 1.0 M H_2SO_4/MeOH was added. The vial was resealed and placed at 100°C for 10 min. The vial was removed from the heating block, cooled to room temperature and the contents extracted into hexane (2 ml). The hexane was washed with saturated brine and then dried over Na_2SO_4. The resultant FAMEs mixture was then injected into the GC under the conditions described previously. Low temperature crystallization of meadowfoam fatty acids by the following method of Chang (1977) provided high purity fractions of oleic, 5-eicosenoic, erucic and 6-octadecenoic acids. Fatty acid (100 g) was dissolved in 2000 ml of acetone and placed in a dry-ice/acetonitrile bath, which provided a constant temperature of −41°C. After 2 h the crystals were filtered through a chilled buchner funnel. The isolated crystals were then allowed to melt into a round bottom flask where the solvent was removed in vacuo. After two crystallizations the monoene purity was >96%. The isolated fatty acids were converted to their methyl esters by refluxing for 90 min in 750 ml of 1.0 M H_2SO_4/methanol solution. The reactions were neutralized with 900 ml of 1.0 M aqueous. KOH and the pH adjusted to 5.0 with NaH_2PO_4 buffer (129.7 g NaH_2PO_4 in 1 l H_2O). The neutralized reaction mixtures were placed into separatory funnels and the FAMEs were extracted into 200 ml of hexane. The hexane layer was then passed through 20 g of basic alumina (Aldrich Chemical, Milwaukee) and concentrated in vacuo. Kugelrohr distillation at 130–160°C at 0.2 mmHg provided the final methyl esters that were free of antioxidants (determined by HPLC). Iodine values for the methyl esters were determined for the FAMEs by GC before OSI testing.

3. Results and discussion

Oxidative stability index (OSI) values were determined on a variety of vegetable oils at 110°C

and these results are reported in Table 1. Of the oils surveyed crude meadowfoam oil gave the best OSI time of 246.9 h. However, refining of the meadowfoam greatly reduced the oxidative stability. Fully refined meadowfoam still performed twice as well as other reportedly stable oils such as jojoba (Table 1). Crude oils have been shown to be more oxidatively stable then their refined counterparts (Akoh, 1994) and has been attributed to a larger amount of tocopherol in the crude oils as compared to the refined oil.

3.1. Oxidative stability of binary oil mixtures

A study was initiated to examine the effect of binary oil mixtures toward oxidative degradation. Table 1 is a list of the parent oils and their respective OSI values. Our results for soybean, refined high oleic sunflower and jojoba oils were consistent with results published by Brown et al. (1997) and Akoh (1994). Table 2 provides the data for the binary mixtures at 5, 10, 20 and 40% blends of parent oil with a more stable oil. Crude meadowfoam oil had the largest impact on the oxidative stability performance of the binary mixtures. Crude meadowfoam oil improved the oxidative stability of technical grade triolein (65% triolein, T-65) by 35% with only a 5% blend and more than doubled the stability at 20%. This triolein contained a significant amount of linoleic acid (6.3%). Refined meadowfoam, however had a much smaller impact requiring a 40% blend to achieve a doubling of the oxidative stability of T-65. Higher purities of triolein gave even more pronounced increases in their oxidative stabilities with 5% blends of crude meadowfoam oil. Triolein 99% had a 19-fold increase with 5% crude meadowfoam oil and triolein 95% had a 21-fold increase in its oxidative stability. High oleic sunflower and crude jojoba oil improved the oxidative stability of triolein 65%, but required > 40% blend to achieve a doubling of the OSI time. Crude meadowfoam had very little effect on the stability of high oleic sunflower or soybean oils. However, crude meadowfoam did improve the oxidative stability of jojoba oil by 39% even at the 5% blend level.

3.2. Role of tocopherol on oxidative stability

The unusual stability of crude meadowfoam oil and its ability to improve the stability of another oil could possibly be attributed to the total amount of antioxidant present within the oil. Akoh (1994) showed that as soybean oil was refined a reduction of tocopherol was observed which resulted in a reduction in the oxidative stability of the oil. Table 3 provides a summary of

Table 3
Tocopherol content and iodine value of parent oils

	Tocopherol (ppm)[b]					Unsaturation (normalized percent)			
Oil[a]	α	β	γ	δ	Total	Monoenes	Dienes	Polyenes	Iodine value[c]
RHOSO[d]	592	4	525		1121	84.8	4.3	0.0	84.4
RSO	91	17	821	17	946	27.9	48.8	5.0	127.0
HEAR	285		402		687	67.4	16.0	10.6	106.4
CMFO	29		534		563	78.0	19.8	0.4	93.3
RMFO	52		425		477	80.1	19.0	0.0	93.3
CCO[d]		236	219		455	79.3	9.0	3.8	88.5
RJO	11		24		35	98.0	0.0	0.0	80.6
T65	127				127	81.8	6.9	0.0	85.5

[a] See Table 1 and Table 2 for abbreviations.
[b] Total tocopherol content measured by HPLC using a 250 mm × 4.6 mm NH_2 column coupled to a UV detector set at 295 nm.
[c] Iodine value determined by GC of FAMEs according to AOCS recommended practice Cd 1c-85.
[d] High oleic sunflower oil had 500 ppm of tocopherol added to the oil by the manufacturer prior to shipment.
[e] Crude crambe oil.

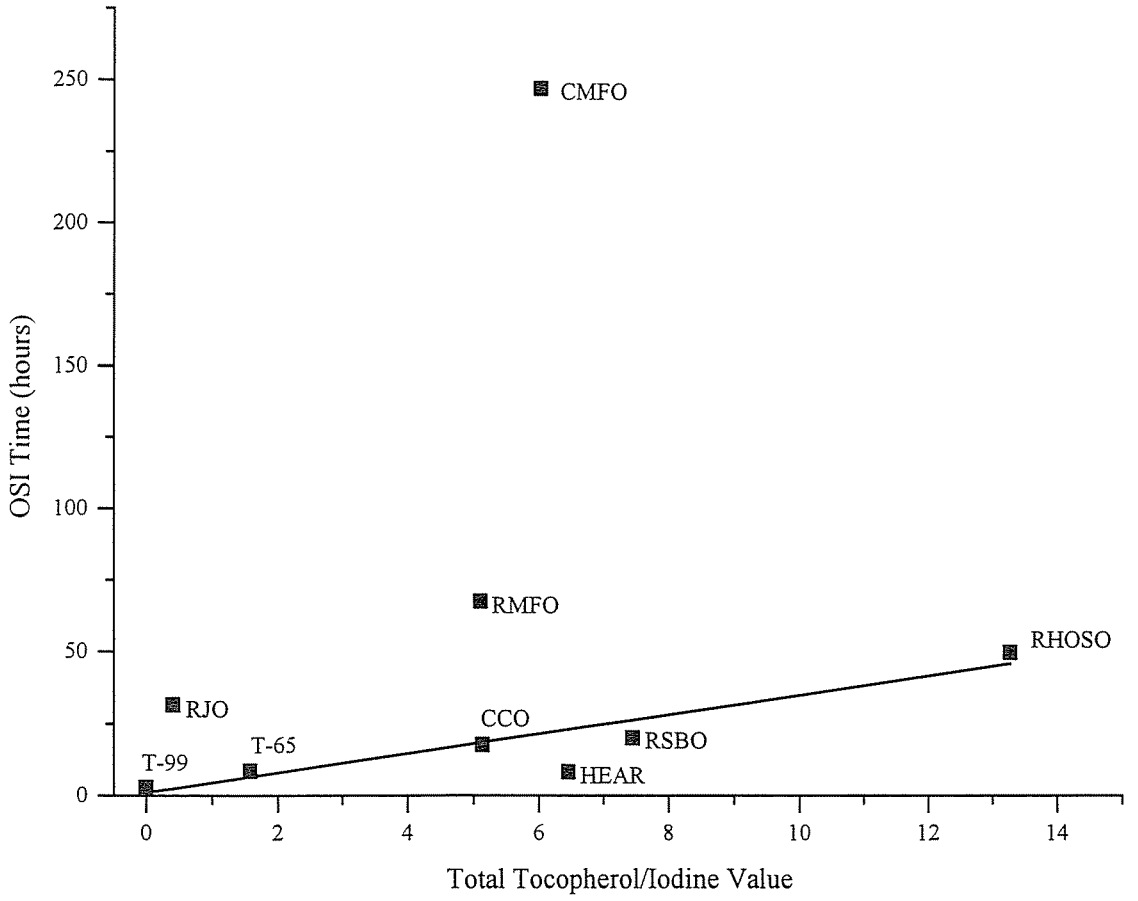

Fig. 1. Relationship of ratio of total tocopherols/IV versus OSI time at 110°C.

the tocopherols present in the oils examined and their respective iodine values. High oleic sunflower had the highest tocopherol contents and this level of anitoxidant is most likely responsible for its large OSI value. Soybean oil also has a significant amount of tocopherol present in the oil, but its large degree of polyunsaturates significantly diminishes its stability. Soybean and HEAR oil have large amounts of polyunsaturates (RSBO 53.8%, HEAR 26.6%), but the HEAR oil has 1/3 less tocopherol present. Consequently, the HEAR oil has an OSI time nearly 1/2 that of refined soybean oil. Even small amounts of polyunsaturates negatively impacted the OSI time under nearly equivalent tocopherol contents. When a plot of the ratio of tocopherol concentration to iodine value against OSI time was made, a linear relationship is observed for oils with similar fatty acid compositions, Fig. 1. Thus, as tocopherol concentration increases and/or IV decreases, the OSI appears to increase in a linear manner for these oils. However, when meadowfoam oil and jojoba wax esters are included, Fig. 1, the relationship becomes much less clear. The results indicate that even when an accounting of tocopherol and degree of unsaturation are taken into consideration, crude meadowfoam oil has an unusually high oxidative stability index. Other factors, including additional antioxidants present, but not characterized in this study, could explain the differences in the observed performance of these oils.

To further define the role of tocopherols in the oxidative stability of crude meadowfoam and its

binary mixtures, a control experiment was performed where triolein was dosed with the appropriate amounts of tocopherols to simulate the dosage that would result from addition of meadowfoam oil in the binary mixtures. Triolein (tech., 65% triolein) was stabilized with 226 ppm tocopherol to simulate a 60:40 triolein/crude meadowfoam oil binary mixture. OSI curves of triolein (tech.), triolein (tech.)/crude meadowfoam 60:40 and the triolein/tocopherol mixtures are shown in Fig. 2. The technical triolein from Sigma has a considerably different OSI time than the triolein from MCB shown in the earlier binary mixture study. The large difference in the OSI time of these two trioleins is due to the presence of tocopherol (127 ppm) in the initial triolein that was absent in the Sigma sample. However, the relative improvement in the triolein's stability is unchanged. The 90:10 mixture of triolein/CMFO clearly outperformed the triolein/tocopherol (226 ppm) mixture which simulates the tocopherol dosage of a 60:40 triolein/CMFO mixture. Therefore, these data clearly indicate that tocopherol alone is not responsible for the high stability of CMFO or the improved stability of its admixtures with other oils.

3.3. Oxidative stability of olefin position

Meadowfoam oil is unique in that the triacylglycerol consists of >95% mixture of fatty acids with carbon chain lengths greater than 18. The majority of these are *cis* Δ5 fatty acids, (≈66%) is 5-eicosenoic acid, (≈16%) 5,13-docosadienoic acid, and (≈11%) 5 and 13-docosenoic acid. Reports of increased oxidative stability of the 5-ei-

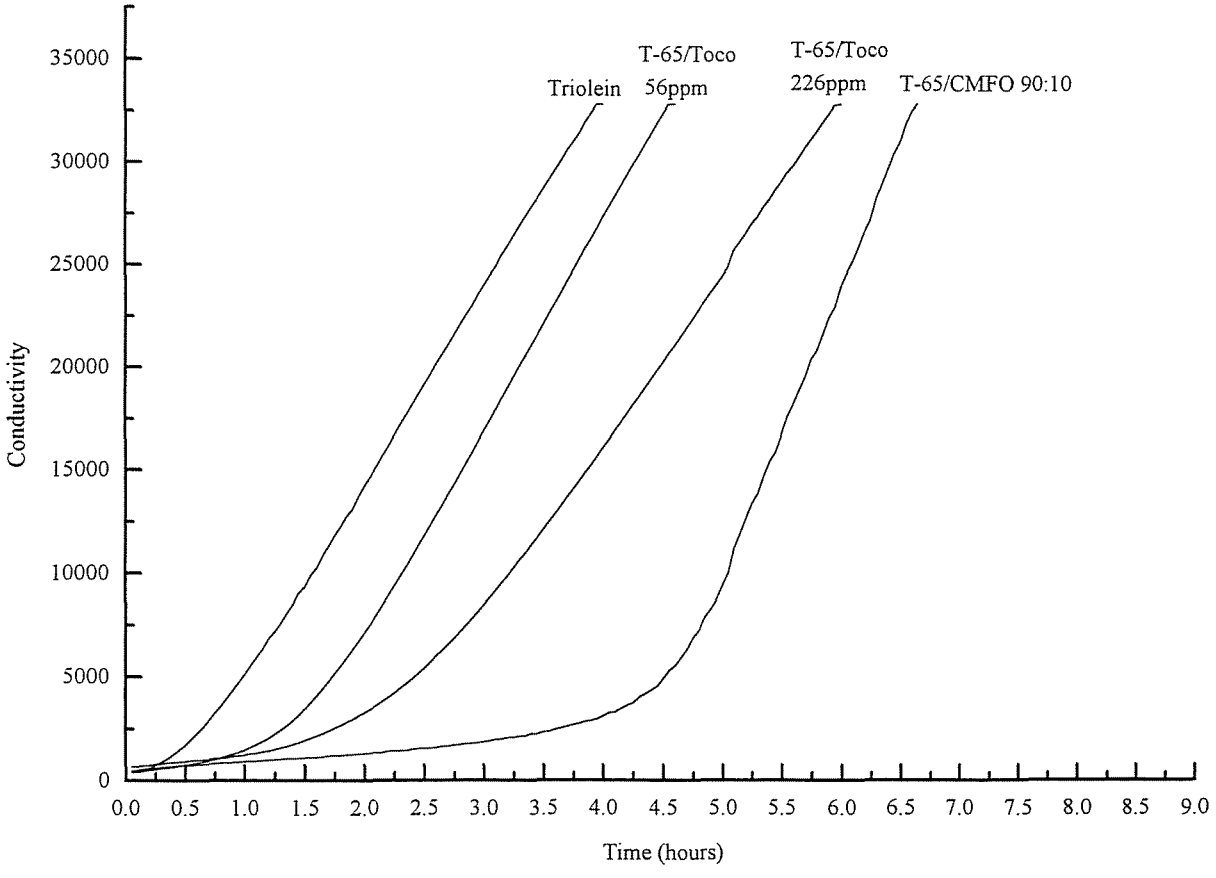

Fig. 2. Oxidative stability at 110°C of triolein (T65) as a function of meadowfoam oil.

Table 4
Oxidative stability index of isolated fatty acid methyl esters at 90°C

Methyl ester	OSI (h)	Iodine value
9-Octadecenoic methyl ester[a]	14.8	91.7
Meadowfoam methyl esters	4.9	90.5
13-Docosenoic methyl ester[b]	4.9	70.6
5-Eicosenoic methyl ester[c]	69.4	79.3
6-Octadecenoic methyl ester[d]	4.9	83.9

[a] 9-Octadecenoic 91.4%, monoenes 95.8%, dienes 0.3% and saturates 3.8%.
[b] 13-Docosenoic 93.0%, monoenes 94.1% and saturates 5.9%.
[c] 5-Eicosenoic 83.3%, monoenes 96.0% and saturates 4.0%.
[d] 6-Octadecenoic 90.6%, monoenes 92.1%, dienes 0.4% and saturates 7.5%.

cosenoic acid have been published by Kaneniwa et al. (1988). However, pure fatty acid fractions have not yet been examined. We compared the methyl esters of meadowfoam to oleic and erucic acids and found that meadowfoam FAMEs performed poorly when compared to methyl oleate, even though they have similar iodine values (Table 4). Erucic acid methyl esters (IV = 70.6) also gave a poor OSI time even though the monoene content was very high. Purification of 5-eicosenoic acid by low temperature crystallization in acetone (Chang and Rothfus, 1977) gave a fatty acid fraction that was 83.3% Δ5 C20:1 and 96% enriched in monoenes. Distillation of this material after conversion to methyl esters gave an OSI time of 69.4 h at 90°C. This ester is nearly an order of magnitude more stable than all the other esters studied. All of the esters in this study were passed through basic alumina and vacuum distilled prior to OSI runs to remove residual free fatty acids and tocopherols. HPLC analysis of the FAMEs prior to OSI testing confirmed the removal of tocopherol. In addition, titration of the FAMEs for acid values (AV < 0.2 mg KOH/g ester) demonstrated a low amount of free fatty acid within the samples. The unusually large OSI time for 5-eicosenoic acid methyl ester suggests that the Δ5 double bond is a unique chemical entity particular with respect to auto-oxidation. This effect appears to be limited to unsaturation nearest the carboxylic functionality (from at least the Δ5 position since isolated petroselenic acid, Δ6 olefin, from coriander oil gave a low OSI time of 4.9 h).

Oleate performed surprisingly well when compared to erucic and petroselenic esters. We do not have an explanation for this observation at present. The 5-position has been shown to react very uniquely in chemical reactions as has been demonstrated in our laboratory (Isbell and Plattner, 1997). The ability to inhibit oxidation of the Δ5 fatty acid may be the result of stereoelectronic repulsion of radical species with the electron rich carboxylate functionality, thus limiting the number of trajectories that lead to initial radical formation.

The Δ5 fatty acid stability must not play too large a role in the overall stability of meadowfoam oil because the meadowfoam FAMEs mixture did not have unusual oxidative stability characteristics. In addition, refining of the oil greatly reduces its oxidative stability, yet the triglyceride composition remains unchanged when processing from crude to refined oil.

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